DETERMINATION OF ITRACONAZOLE AND ITS METABOLITE FROM HUMAN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Itraconazole is used for the treatment of fungal infections in both HIV and non-HIV-infected individuals. Itraconazole is administered in its active acid form and gives very low plasma concentrations (ng/ml levels). Liver metabolism produces active hydroxy metabolites.

A simple and sensitive high performance liquid chromatographic method with tandem MS detection (HPLC-MSMS) for the determination of itraconazole and its hydroxy metabolite from plasma was developed and validated. Liquid-liquid extraction was used for extracting itraconazole and its metabolite from plasma. Hegzan:isoamylalcohol (97:3) was used as extraction solvent. The chromatographic separation of itraconazole, metabolite and ketoconazole (IS) was carried out using reverse phase Water X-terra RP-C18 column (150x4.6 mm, 3.5μm) with mobile phase of Acetonitrile:Ammonia (0.1 %) 80:20 (v/v). The flow rate of mobile phase was 0.4 ml/min, injection volume was 15 μl. The mass spectrometric parameters were optimized to obtain maximum sensitivity at unit resolution. Electrospray mode(-ES) was used at positive ionization. The ions used to quantify were selected as parent to daughter m/z 705.30→392.30 for itraconazole, m/z 721.25→408.30 for hydroxymetabolite and m/z 531.20→489.15 for IS. The calibration curve was linear within the concentration range 1-600 ng/ml and 2-600 ng/ml for itraconazole and metabolites respectively. The stability was assessed under a variety of conditions and found that appropriate for the quantification. The method developed can be used for bioequivalence and pharmacokinetic studies.

References